American Scientific Research Journal for Engineering, Technology, and Sciences (ASRJETS)
ISSN (Print) 2313-4410, ISSN (Online) 2313-4402

http://asrjetsjournal.org/

Antioxidant and Antifungal Activities of Cocoa Butter (*Theobroma cacao*), Essential Oil of *Syzygium aromaticum* and a Combination of Both Extracts against Three Dermatophytes

Patience Mekemzeu Fankem[a], Sylvie Nguikwie Kwanga[b], Modeste Lambert Sameza[c], François Tchoumbougnang[d], Raymond Tchabong[e], Leopold Tatsadjieu Ngouné[f], Pierre Michel Jazet Dongmo[g*]

[a,b,c,e,g]*Department of Biochemistry, Faculty of science, University of Douala, P.O. Box 24157, Douala, Cameroon*

[d]*Fisheries and Halieutic Science Institute, University of Douala, P.O. Box 7236, Douala, Cameroon*

[f]*Department of Food Engineering and Quality Control, University Institute of Technology, University of Ngaoundere, P.O. Box 455, Ngaoundere, Cameroon*

[g]*Email: mjazet@yahoo.com*

Abstract

To contribute in the research of better drugs against dermatophytosis, we evaluated the antioxidant and antidermatophytic activities of cocoa butter, cloves essential oil, and a mixture of both extracts. The cocoa butter was obtained by boiling the cocoa paste. The essential oil extracted by hydrodistillation was chemically analysed by gas chromatography and gas chromatography coupled with mass spectrometry. The antioxidant activity was determined using the DPPH scavenging method, and the antidermatophytic activity was evaluated using the agar dilution method. The essential oil, majoritary constituated by eugenol (87.62%), β-caryophyllene (5.88%), and β-bisabolene (4.41%), had an antiradical power (4.22×10^{-2}) higher than that of BHT (4.00×10^{-3}), like the cocoa butter and essential oil mixture (6.06×10^{-3}). The essential oil was more active than the griseofulvin: it was fungicidal at 400 ppm against *Trichophyton rubrum*, and at 900 ppm against *Microsporum gypseum*and *Trichophyton tonsurans.*

---

* Corresponding author.

The cocoa butter activity was low, but the mixture with the essential oil had an important activity with inhibitory percentages of 78.69 %, 88.27 %, 91.20% against *T. rubrum* (at 400 ppm), *T. tonsurans*(at 900 ppm)and *M. gypseum* (at 900 ppm)respectively. Cloves essential oil and the mixture with cocoa butter can be used to formulate new drugs against dermatophytes.

Key words: Antioxidant; antidermatophytic; cocoa butter; cloves; essential oil.

1. Introduction

Infectious diseases, especially dermatophytosis, are a major threat to human health [1,2,3]. The incidence and mortality from these infections are influenced by the characteristics of the population at risk, the availability of medical care, distribution of species responsible and prevalence of antimicrobial resistance [4,5]. For centuries, the treatment of diseases was done with different formulations and extracts of plants for their medicinal properties. Thus, fungal infections have been successfully managed using medicinal plants [6,7,8,9]. Moreover, infectious diseases are treated by modern medicines such as antibiotics and antifungals. However, these drugs are not always accessible to poor communities in developing countries [10]. In addition, most of these drugs have low antimicrobial spectrum, a long duration of treatment, side effects, and their wide spread excessive use leads to resistance of microorganisms. For example, ketoconazole previously used in the treatment of some dermatophytes causing ringworm is now rarely used in case of severe systemic fungal infection, because of its hepatotoxic effects [11]. The upsurge of the resistance of fungal strains is one of the barriers to the successful treatment of microbial diseases. It increased the universal demand for herbal medicine that is now an integral part of primary care in most countries [12]. Dermatophytic infections are associated with many oxidative reactions that may be responsible of the production of free radicals, which contribute to the increase of body lesions. Meanwhile, synthetic antioxidants usually used have side effects for the organism (the Butylated hydroxytoluene is a carcinogen molecule). Therefore, it is essential for man to find an alternative to these treatments. Plants have bioactive metabolites (alkaloids, saponins, flavonoids, tannins and phenolic compounds), which are responsible for their therapeutic potential [13]. These are a good source of anti-infective agents, and antioxidants and are a natural reservoir of new biologically active molecules to be discovered. *Theobroma cacao* known as cocoa, is a plant whose products are widely used: the chocolate for its organoleptic, nutritional and stimulant qualities and cocoa butter, used in pharmacy and cosmetics primarily for its moisturizing, nourishing and antiseptic properties on the skin and hair [14]. Besides this plant, *Syzygium aromaticum*, commonly called clove, is an aromatic plant used in traditional medicine because of its many medicinal properties. It has certainly been the subject of several scientific works [15,16,17,18,19], but few of these works have addressed antidermatophytic properties of the plant essential oil.

2. Material and methods

2.1. Vegetable material

Cocoa capsules and dried cloves buds (*Syzygium aromaticum*)were collected at Penja**,** on June and July 2016 respectively.

These plants were identified at the National Herbarium of Cameroon, in Yaounde, with references number of cocoa and clove trees being 35970TW/CAM and 2008SKR/CAM.

2.2. Fungal material

Two referenced dermatophytes: *Microsporum gypseum*(E1420), *Trichophyton rubrum* (BDO23) and one isolate: *Trichophyton tonsurans* were studied. They were obtained from the Laboratory of Microbiology and Antimicrobial Substances of the University of Dschang.

2.3. Hydrodistillation

The best way to extract clove essential oil was the hydrodistillation [16,20,21].

250g of clove buds were macerated into a liter of water for 8 hours and then introduced in a Clevenger apparatus [22] for hydrodistillation for 8 hours.

Two phases were obtained: an organic phase, which was the essential oil, and a water-soluble phase, constituted by water and essential oil. The essential oil was separated from water in the second phase using hexane, by a liquid-liquid extraction [16]. The hexane was evaporated through a rotary evaporator at 80°C, and the purified essential oil was added to the previous organic phase. The essential oil obtained was dried using anhydrous sodium sulphate and kept it in a refrigerator at 4°C. The extraction yield was calculated by the following formula:

$$\textbf{Yield (\%)} = \frac{\textbf{weight of essentialoil (g)}}{\textbf{weight of vegetable material (g)}} \times \mathbf{100}$$

2.4. Extraction of cocoa butter

The cocoa capsules were opened; all the beans were removed and fermented for 5 days, then dried in a sterilizer at 50°C for 12 hours. The dried beans were roasted, and their husk removed and mashed to obtain a paste. 2500g of cocoa paste were cooked with 5L of water in order to obtain the cocoa oil as described by [23]. The oil was filtered, treated three times with distillated water to keep out remains, and completely dehydrated with anhydrous sodium sulphate. The storage was in a dark and dry place. At room temperature, the oil solidified and became cocoa butter.

The extraction yield was calculated by the following formula:

$$\textbf{Yield (\%)} = \frac{\textbf{weight of cocoa oil (g)}}{\textbf{weight of cocoa paste (g)}} \times \mathbf{100}$$

2.5. Chemical composition analysis of the essential oil

The essential oil was analyzed by gas chromatography and gas chromatography coupled with mass spectrometry [24].

2.5.1.Gas chromatography (GC)

The oil was analyzed on a chromatographic Variant type CP 3380, equipped with a flame ionization detector and a fused silica capillary column (30 m x 0.25 mm) coated with a DB5 film thickness of 0.25µm. The operating conditions were as follows; the temperature of the injector and detector was programmed at 200℃; the temperature of the oven from 50℃ to 200℃at 5℃/min; with nitrogen gas vector of 1mL/min. The linear retention indices of the components were determined relatively by the retention time of a series of n-alkanes and the percentage compositions were obtained from electronic integration measurements without taking into account relative response factors.

2.5.2. Gas Chromatography/Mass Spectrometry (GC/SM)

GC/MS analysis was performed using a Hewlett-Packard apparatus equipped with an HP1 fused silica column (30m x 0.25mm, film thickness 0.25µm) and interfaced with a quadrupole detector (GC- quadrupole MS system, model 5970). Column temperature was programmed from 70 -200°C at 10°C/min; injector temperature was 200°C. Helium was used as a carrier gas at a flow rate of 0.6 ml/min. The mass spectrometer was operated at 70eV.

2.6. Identification of the essential oil components

The qualitative analysis was made possible by calculating the Kovats index (KI) of each element, based on their retention time and the retention time of a set of alkanes used as a standard. Thus, the identification was assigned by the comparison of the KI with those given by literature, and with the stored laboratory mass spectral library [24].

2.7. Evaluation of the antiradical activity

The antiradical activity of the cocoa butter, the essential oil and the mixture of both substances were evaluated by measuring the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) scavenging power at 517 nm [25]. Butylated hydroxytoluene (BHT) was used as the reference antioxidant. 20 mg of DPPH and 50 mg of BHT were dissolved in methanol, in order to obtain a 40mg/L and a 1g/L solution, respectively**.** Hexane was use to mix essential oil and cocoa butter. The negative control solution was represented by the DPPH methanolic solution. The final volume of solution in all the tubes was 2200 mL (**Table 1**). The optical densities were read at 517 nm using a spectrophotometer [26], after 2 hours at room temperature. The following parameters were determined:

- The $\mathbf{SC_{50}}$ (50% Scavenging Concentration) was determined graphically: it is the concentration required for 50% DPPH reduction [27].

- The $\mathbf{EC_{50}}$ **(**Effective Concentration), in grams of extract per mol of DPPH.

Table 1: Evaluation of the antiradical activity of BHT, cloves essential oil (EO), cocoa butter (CB), and the mixture (EO + CB)

Tested substances	Essential oil (EO)	Cocoa butter (CB)	Mixture (EO + CB)
Concentration of the solution in standard tubes (mg/L)	25, 50, 100, 200, 400	1000, 2000, 3000, 4000, 5000	- EO : 25, 50, 100, 200, 400 - CB : 5000
Volume of tested substance introduced in standard tubes (µL)	100	200	- - EO : 100 - CB : 100
Volume of DPPH per tube (µL)	2000	2000	2000
Volume of BHT in the reference tube (µL)	100	200	200

Reference tube: tube containing only BHT and DPPH.

CE_{50} (g of extract/ mol of DPPH) = SC_{50}/ C_{DPPH},(C_{DPPH}: Molar Concentration of DPPH in each

- The **AP (**Antiradical Power): expresses the antioxidant as the highest and most effective.

2.8. Evaluation of the antifungal activity

The antifungal activity was performed using three dermatophytes: *Microsporum gypseum*, *Trichophyton rubrum* and *Trichophyton* tonsurans, following the agar diffusion method [28].

Samples were prepared with the Sabouraud Dextrose Agar supplemented with chloramphenicol (SDA+ chloramphenicol) medium, the EO, and the cocoa butter, in a scale of several concentrations. The Dimethyl Sulfoxyde (DMSO) was used as the surface acting in essential oil samples, and the Tween 80 in cocoa butter samples.

Griseofulvin was used as the reference antifungal, and the negative control was represented by the DMSO in SDA medium. Each sample was prepared three times.

2.8.1. Antifungal activity of the essential oil of Syzygium aromaticumand cocoa butter

A solution was firstly prepared with the essential oil and the DMSO in 1/9 (v/v) proportion; then, different volumes of the solution were added in the liquid SDA, in order to obtain different concentrated solutions with a final volume of 10mL.

The initial concentration scales were: 500, 750, 1000, and 2000 ppm; these concentrations were successively split to determine the accurate Minimal Inhibitory Concentration (MIC) of the essential oil.

The cocoa butter was treated using the same protocol, but the first solution was prepared with CB and tween 80, at 2.5% of tween, and the initial concentration scales were: 500, 750, 1000, 2000 and 4000 ppm.

All samples were poured in 90 mm Petri dishes, and a mycelium explant of 2 mm was deposited in the middle of each dish. The dishes were sealed and incubated at room temperature in their inverted position.

2.8.2. Antifungal activity of the essential oil and cocoa butter formulation

The antidermatophytic activity of the essential oil and cocoa butter formulation was carried out during 18 days of incubation, at 25°C. The two extracts were dissolved in SDA medium at the same concentration (the chosen concentration corresponded to the Minimal Fungicidal Concentration of the essential oil depending on the germ).

2.8.3. Evaluation of mycelium growth inhibition

The mycelial growth was followed by measuring every two days, and at the same hour, two perpendicular diameters on each Petri dish on the explant level.

The comparison between the mycelial growth in dishes containing antifungal substances and the control dishes helped us to evaluate the radial inhibition of the mycelium, and calculated the inhibition percentage by the following formula:

$$\textbf{Inhibition Percentage} = \frac{\mathbf{Dc-De}}{\mathbf{Dc}} \times \mathbf{100}$$

- Dc (cm) = mycelium growth diameter in the control,
- De (cm) = mycelium growth diameter in the dish which contained an antifungal.

The Minimal Inhibition Concentration (MIC) was determined.

2.8.4. Nature of inhibition

The fungicidal or fungistatic activity was evaluated by the transfer of explants from the dishes containing the medium combined with EO at the MIC, into a sterile medium.

The substance was fungistatic when the dermatophyte had grown in the new medium during the incubation period, and it was fungicidal when no mycelium growth was observed.

2.9. Statistical data analysis

The numerical data were introduced in EXCEL (Microsoft, 2010). The data was analyzed with the Stat view software version 5.0 (SAS Institute Inc., USA).

A one-factor Analysis of Variance (ANOVA) and the non-parametric test of Kruskal-Wallis were used, and the significance level was below the probability of 0.05.

3. Results

3.1. Essential oil and cocoa butter characteristics

The extracted essential oil and cocoa butter showed different characteristics (**Table 2**).

Table 2: Essential oil and cocoa butter characteristics

	Vegetable material(g)	**Product (g)**	**Yield (%)**	**Color**
Essential oil	1000	96.6	9.66	Pale yellow
cocoa butter	2500	400	16	Yellow

3.2. Chemical composition of the essential oil

The essential oil of *S. aromaticum* of Penja in Cameroon contained 28 constituents **(Table 3)**, mainly oxygenated monoterpenes (89.06%) and hydrocarbonated sesquiterpenes (10.86%). Three main constituents were present: eugenol (87.62 %), β-caryophyllene (5.88 %) and β-bisabolene (4.41 %).

3.3. Antiradical Potential of BHT and Syzygium aromaticum essential oil

The absorbance values read on the spectrophotometer at 517 nm were used to calculate the scavenging percentages of the radical DPPH by the EO, which were graphically used to determine the EO Scavenging Concentration 50 which was equal to 2.26×10^{-3} g/L (**figure 1**).

3.4. Antiradical potential of cocoa butter

The maximal scavenging percentage of cocoa butter was lower than 10% (9.57 ± 1.16%) at 0.73 g/L concentration **(Table 4)**.

Table 3: Chemical composition of *Syzygium aromaticum* essential oil.

Index	KI	Components	Quantity (%)
Monoterpenes			**89.10**
Hydrocarbonatedmonoterpenes			**0.04**
1	1019	P- cymene	0.01
2	1087	terpinolene	0.03
OxygenatedMonoterpenes			**8.06**
3	1027	1,8- cineole	0.03
4	1079	fenchone	0.03
5	1104	camphenol	0.03
7	1212	carveol	0.01
8	1237	geraniol	0.35
9	1353	eugenol	87.62
10	1367	dihydro-eugenol	0.21
11	1445	isoeugenol	0.67
12	1521	eugenylacetate	0.05
Sesquiterpenes			**10.87**
Hydrocarbonatedsesquiterpenes			**10.6**
13	1338	δ-elemene	0.09
14	1412	β-caryophyllene	5.88
15	1463	epi-(E)- Caryophyllene	0.05
16	1465	γ-gurjunene	0.02
17	1483	germacrene-D	0.02
18	1489	β-selinene	0.08
19	1511	β-bisabolene	4.41
20	1514	Δ-cadinene	0.05
Oxygenatedsesquiterpenes			**0.27**
21	1545	Elemol	0.02
22	1576	spathulenol	0.14
23	1602	Guaiol	0.02
24	1618	cubenol	0.04
25	1627	γ-eudesmol	0.01
26	1674	β-bisabolol	0.04
Linear components			**0.03**
27	844	3(E)-hexenol	0.01
28	884	4-heptanol	0.02

KI :Kovats Index

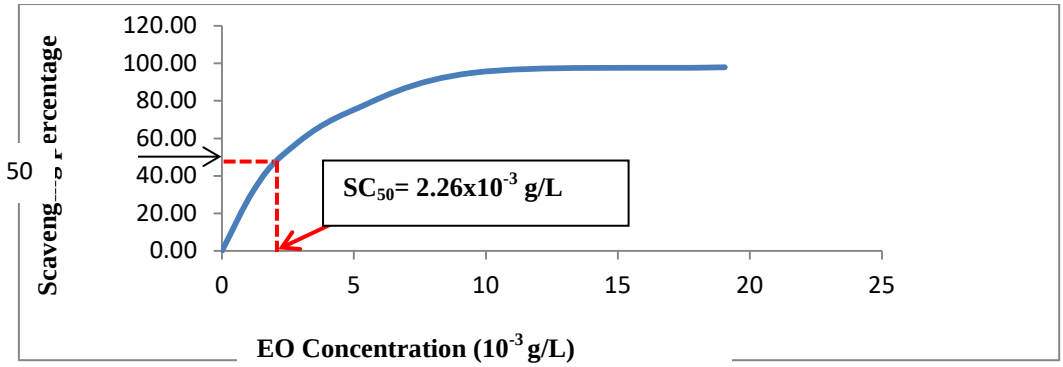

Figure 1: Variation of scavenging percentage in function of the EO concentration

The SC_{50} of BHT was graphically determined to be 2.27 **x** 10^{-2} g/L.

Table 4: Scavenging percentages of the cocoa butter

CB concentration (g/L)	**0**	**0.045**	**0.09**	**0.18**	**0.36**	**0.73**
% de piégeage ± SD	0.00	0.95 ± 0.26[a]	5.92 ± 0.29[b]	6.37 ± 0.18[b]	6.98 ± 0.00[c]	9.57 ± 1.16[d]

***SD**: Standard Deviation; **a, b, c, and d** show the significant difference between scavenging percentages at different concentrations of CB: two concentrations have the same letter if there is no significant difference between scavenging percentage values.*

3.5. Antiradical potential of the essential oil and cocoa butter mixture

Figure 2 shows the variation of the scavenging percentages of DPPH according to the essential oil and cocoa butter mixture. This plot allowed the determination of the SC_{50} of the (CB+EO) mixture, whose value was 15 x 10^3 g/L.

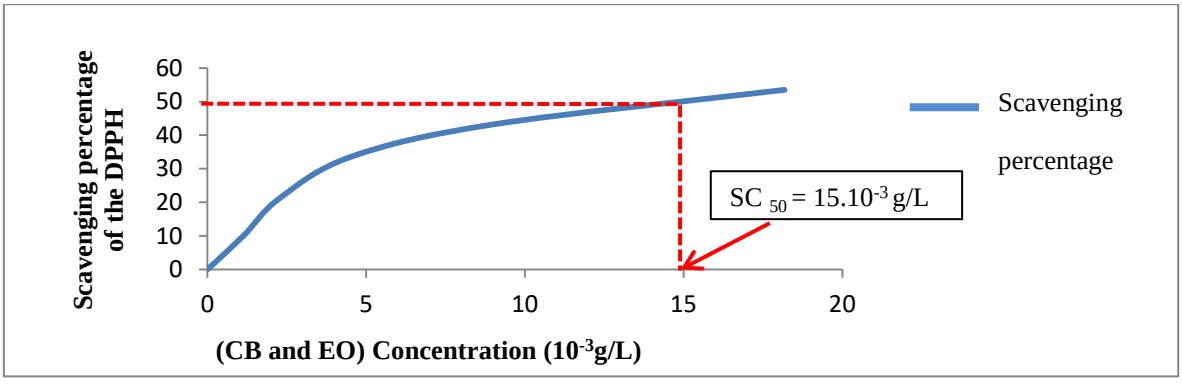

Figure 2: Variation of scavenging percentage of DPPH with respect to EO and CB concentration.

The 50% Efficacy Concentration and the Antiradical Power of each studied substance were calculated using the SC_{50} corresponding values **(Table 5)**.

Table 5: SC_{50}, EC_{50} and AP of BHT, EO, CB and (CB+EO) mixture

Tested substance	SC_{50} (g/L)	EC_{50} (g of EO /mol of DPPH)	AP
***S. aromaticum*essential oil**			
	2.26×10^{-3}	**23.70**	**4.22×10^{-2}**
Cocoa butter	ID	ID	ID
(CB+EO) mixture	$15. 10^{-3}$	165	6.06×10^{-3}
BHT	2.27×10^{-2}	249.70	4.10^{-3}

ID= Indeterminate

The comparison of the SC_{50}, CE_{50} and AC values by the Kruskal-Wallis test made with the Statview software version 5.0, showed that they each had a significant difference (H= 8.000 ; p= 0.0460).

3.6. Antidermatophytic activity of the esssential oil

M. gypseum and *T. Tonsurans* growth was completely inhibited from 1000 ppm, while *T. rubrum* growth was completely inhibited at all the chosen concentrations (500, 750, 1000 and 2000 ppm). Based on these results, intermediate concentrations were defined to determine the accurate MIC value against each dermatophyte.

The data statistical analysis revealed that:

- The inhibition percentages of the EO at various concentrations were significantly different;
- *T. rubrum* growth was not influenced by the incubation duration;
- The incubation duration significantly influenced *T. tonsurans* and *M. gypseum* until the 18^{th} and 20^{th} day of incubation respectively.

Figure 3 illustrates the variation of the mycelium growth inhibition percentage depending on the EO concentration, after 18 days of incubation.

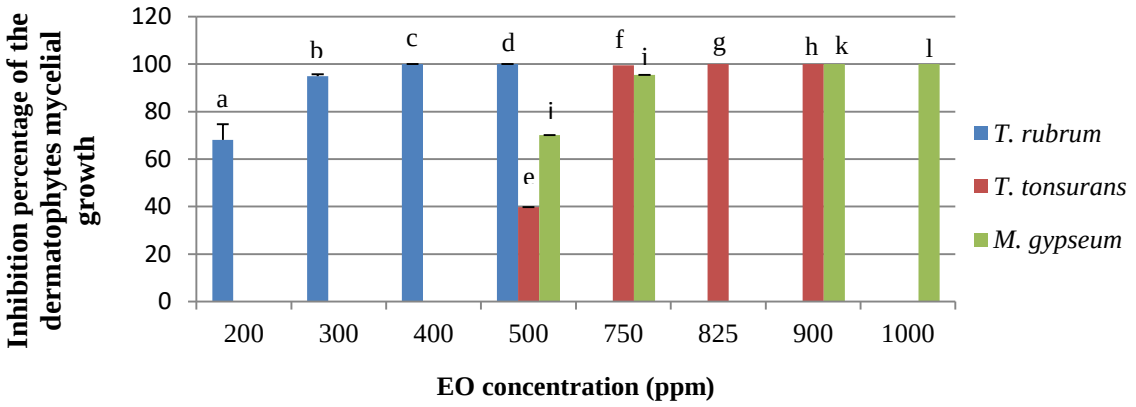

Figure 3: Variation of the mycelial growth inhibition percentage depending on the EO concentration, after 18 days of incubation

On the 18th incubation day, *T. rubrum* was totally inhibited (100% of inhibition percentage) at 400 ppm and beyond, while the case was observed for *T. tonsurans* and *M. gypseum* from 825 and 900 ppm respectively.

There was no growth of *T. rubrum* during the incubation time in Petri dishes which contained essential oil at 400 ppm. Up to the last incubation day, no growth of *T. tonsurans* was observed in Petri dishes which contained essential oil at 825 ppm. No grow was observed with *M. gypseum* in Petri dishes where the essential oil concentration was equal to 900 ppm.

3.7. MIC and MFC Determination

The MIC values of *Syzygium aromaticum* essential oil from Cameroon were 400, 825 and 900 ppm, respectively against *T. rubrum*, *T. tonsurans* and *M. gypseum*.

Explants were taken from Petri dishes containing essential oil at the MIC, put in new dishes containing only sterile medium, and the whole was incubated at most during 18, 20 and 26 days for *T. tonsurans*, *M. gypseum*, and *T. rubrum* respectively. Following that, conclusions about the antidermatophytic activity of the cloves essential oil were that:

- *Syzygium aromaticum* essential oil was fungicidal against *M. gypseum* and *T. rubrum* respectively at 900 and 400 ppm;
- *Syzygium aromaticum* essential oil was fungistatic against *T. tonsurans* at 825 ppm (the explant growth was recovered after two incubation days in the new medium). But the MCF was found at 900 ppm concentration.

The comparison of those MIC and MFC values by the Kruskal-Wallis test confirmed their significativity at a level of 0.05%.

3.8. Antidermatophytic activity of cocoa butter

The incubation duration of each germ in the medium containing cocoa butter was the same as in the medium with the EO, and mycelial growth was as shown in **figure 4**.

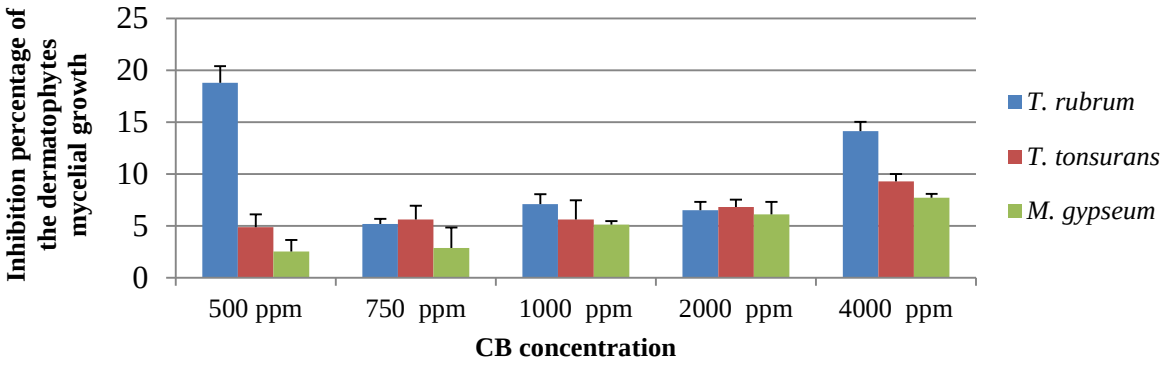

Figure 4: Inhibition percentages of the dermatophytes growth depending on CB concentration, after 18 incubation days.

The maximal inhibition of cocoa butter against *T. rubrum* was observed at 500 ppm (18.8%), followed by the dose of 4000 ppm (14.14%). On the other hand, its maximal inhibition against *M. gypseum*and *T. tonsurans*, (7.72 and 9.28% respectively) was reached with the dose of 4000 ppm.

3.9. Antidermatophytic activity of essential oil and cocoa butter formulation

To formulate the mixture, the essential oil and cocoa butter were introduced in the growth medium at the same concentration, which corresponded to the MFC of the essential oil against the respective dermatophyte. With respect to *T. rubrum*, the extracts were introduced in the medium at 400 ppm each. But for *T. tonsurans* and *M. gypseum*, they were mixed at 900 ppm each. Although the essential oil and cocoa butter formulation did not completely hinder the growth of the three dermatophytes, its inhibitory action was very important. In effect, the mixture had a depressive action on the dermatophytes, because it hindered their growth at least for 10 days for *M. gypseum*, and 12 days for *T. tonsurans* and *T. rubrum* (**figure 5**).

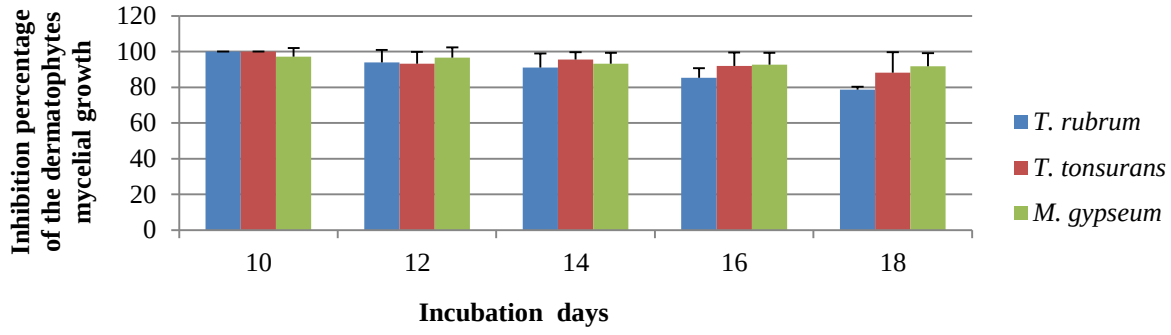

Figure 5: Variation of the inhibition percentage of the dermatophytes growth, depending on the concentration of (EO+CB) mixture during 18 days of incubation.

The inhibition of *T. rubrum* by the (EO+CB) mixture at 400 ppm is higher than 70% after 18 days of incubation (78.69 ± 1.64%). Furthermore, at 900 ppm and after 18 days of incubation, the mixture induced inhibition of 91.20 ± 7.34% and 88.27 ± 11.43% against *M. gypseum* and *T. tonsurans* respectively.

3.10. Antidermatophytic activity of the griseofulvin

The inhibition percentages of the three dermatophytes mycelial growth by the griseofulvin (antifungal reference) after 18 days of incubation are presented on **figure 6**.

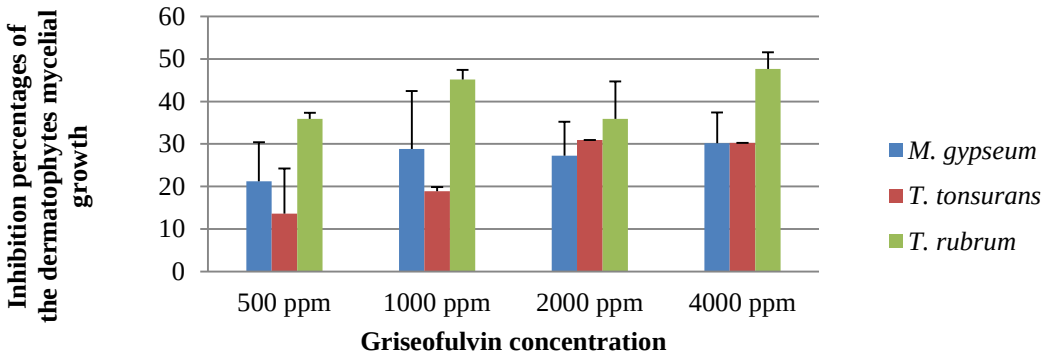

Figure 6: Inhibition Percentages of the three dermatophytes mycelial growth depending on the concentration of the griseofulvin after 18 days of incubation.

The inhibition of each dermatophyte's mycelial growth by the griseofulvin was not total since 100% inhibition was not achieved at any dose level tested (500, 1000, 2000, 4000 ppm).

Griseofulvin was more active against *T. rubrum*, with an inhibition percentage of 47.65 ± 3.89% after 18 days of incubation. Then comes *T. tonsurans* with 30.3 ± 0%, and finally, *M. gypseum* with 30.18 ± 7.22%.Thus, the griseofulvin MIC against the three germs was greater than 4000 ppm.

4. Discussion

The essential oil yield of extraction (9.66%) is different from those obtained by other authors [29,30]who also used hydrodistillation. In effect, it was more than twice greater than the yield of 3.5% obtained by an author [16] after the distillation of dried clove buds collected at Tizi Ouzou. This yield was also far greater than 0.18% obtained with cloves from Benin [15]. As a result of this yield, clove buds essential oil from Cameroon can easily be available.

Many factors can explain these differences: the plant origin, the plant age, the development stage of the plant at the harvest [31], the harvest period, the plant treatment after harvest (drying for example), or the methods and conditions of essential oil extraction [7].

The high amount of eugenol in *S. aromaticum* essential oil was in accordance with the results of several authors

[32, 33,34]. Some scientists showed eugenol as the main component of *S. aromaticum* essential oil, followed by β-caryophyllene [32]. Another one [35] obtained beyond eugenol about 80.8% and β-caryophyllene (10.5%), two other main components: eugenyl acetate (4.4%) and α-humulene (1.26%). Likewise, some authors compared the chemical composition of three clove essential oil samples coming from Madagascar, Indonesia and Zanzibar [19]. They found that each pattern withdrew 10 components, mainly eugenol, β-caryophyllene and eugenyl acetate.

A study of cloves essential oil from Benin [15], showed 21 components for 99.4%, among which oxygenated monoterpenes and hydrocarbonated sesquiterpenes were the principal components: eugenol (60.4%), trans β-caryophyllene (24.0%), eugenyl acetate (10.0%), γ-muurolene (1.4%) and β-sesquiphellandrene (1.7%). These difference can be explained by the lowest extraction yield of the EO of Benin (0.18%), compared to the one of Cameroon clove buds (9.66%), which was far higher.

These results showed that from one region to another, there are many chemotypes of clove buds [7], though eugenol and β-caryophyllene were always found as the traces of cloves essential oil. The cloves essential oil had the strongest antiradical activity (SC_{50}=2.26. 10^{-3} g/L, EC_{50}= 23.7g of EO/mol of DPPH and AP= 4.22 x 10^{-2}). This result was better than the one obtained by some authors [36,37]. This high antiradical activity could be the fact of the high rate of eugenol (87.62%) in the essential oil from Cameroon. In effect, some authors prooved an important antiradical activity of eugenol [38,39,40,41]. In fact, the EO antiradical capacity was ten times greater than the BHT sample and seven times greater than the CB and EO mixture sample. Moreover, the ratio of the mixture antiradical on that of BHT was 1.5, showing a higher antiradical potential of the CB and EO mixture. From the antidermatophytic activity, the essential oil of *Syzygium aromaticum* was more potent than griseofulvin, the reference antifungal, with MICs of 400 p pm against *T. rubrum*, 825 ppm against *T. Tonsurans* and 900 ppm against *M. gypseum*. That activity confirmed the antifungal property mentionned by an author [39]. On the other hand, cocoa butter was weakly active, compared to griseofulvin. The supplementation of cocoa butter by *Syzygium aromaticum* essential oil increased its antidermatophytic activity against the three studied dermatophytes, and the (EO + CB) mixture was more active than the griseofulvin. Of the three products (EO, CB and (EO+CB) mixture), we had shown that the essential oil was the most active, and its activity far exceeded that of griseofulvin, regardless of the considered dermatophyte.

5. Conclusion

The essential oil of *Syzygium aromaticum*is a good natural source of antioxidants, and provides a compounding basis for the treatment of dermatophytosis. Otherwise, cocoa butter can be used successfully to formulate antidermatophytic drugs that include the essential oil of *Syzygium aromaticum*.

Acknowledgement

Special thanks to the BiochemistryLaboratory of the Microbiology and Antimicrobial Substances (LAMSA) of the Department of Biochemistry of the Faculty of Science of the University of Dschang; and the laboratory of Max Mousseron Biomolecules Institute of the University of Montpellier II.

References

[1] A.J.G. Araújo, M.A.J. Souza, O.M. Bastos, J.C. Oliveira. "Occurrence of onychomycosis among patients attended in dermatology offices in the city of Rio de Janeiro, Brazil". Anais Brasileiros De Dermatologia journal, vol 78, pp. 299-308, 2003.

[2] A. Sepahvand, J. Abdi, Y. Shirkhani, Sh. Fallahi, M. Tarrahi, S. Soleimannejad. "Dermatophytosis in Western Part of Iran, Khorramabad". Asian Journal of Biological Sciences, vol 2, pp. 58-65, 2009.

[3] E.R. Siqueira, J.C. Ferreira, C.M.L. Maffei, R.C. Candido. "Occurrence of dermatophyte, in nails, feet and hands of universitary students". Revista da Sociedade Brasileira de Medicina Tropical, vol 39, pp.269-71, 2006.

[4]V. Arya, S. Yadav, S. Kumar, J.P. Yadav. "Antimicrobialactivity of Cassia occidentalis L. (leaf) againstvarioushumanpathogenic microbes". Life science and medicalresearch journal, vol 9, pp. 1-11, 2010.

[5] P.N. Chowdhry, S.L. Gupta, N. Anand. "Diversity of Fungi as Human Pathogen".Recent Research in Science and Technology, vol 5, pp. 17-20, 2013.

[6] T. Jiofack, C. Fokunang, N.M. Guedje, V. Kemeuze, E. Fongnzossie, B.A. Nkongmeneck, et al. "Ethnobotanical uses of medicinals plants of two ethnoecological regions of Cameroon". International Journal of Medicine and Medical Sciences, vol 2, pp. 60-79, 2010.

[7]K.R.Park, D. Nam, H.M. Yun, S.G. Lee, H.J. Jang, G. Sethi, et al. "Beta-caryophyllene oxide inhibitsgrowth and induces apoptosis through the suppression of PI3K/AKT/mTOR/S6K1 pathways and ROS-mediated MAPKs activation". Journal of cancer letters, vol 312, pp. 178-88, 2011.

[8] E. Pinto, L. Vale-Silva, C. Cavaleiro, L. Salgueiro. "Antifungalactivity of the clove essential oil from Syzygium aromaticumon Candida, Aspergillus and dermatophyte species". Journal of Medicine and Microbiology, vol 58, pp. 1454-462, 2009.

[9] D. Webster, P. Taschereau, R. J. Belland, C. Sand, R. P. Rennie. "Antifungal activity of medicinal plant extracts; preliminary screening studies". Journal of Ethnopharmacology, vol 115, pp. 140-46, 2008.

[10] J.C.N. Assob, H.L.F. Kamga, D.S. Nsagha, A.L. Njunda, P.F. Nde, E.A. Asongalem, et al. "Antimicrobial and toxicological activities of five medicinal plant species from Cameroon Traditional Medicine in BMC". Complementary and Alternative Medicine, vol 11, pp. 70, 2011.

[11] Y. Kongo-Nzuzi. "Evaluation in vitro des pouvoirs antifongiques des extraits des feuilles de papayer sur des souches de Candida albicans".Graduéeen techniques de laboratoire, Institut Supérieur des Techniques Médicales, Kinshasa, 2009.

[12] A.D. Afagnigni. "Evaluation des activités antibactériennes et antifongiques de Dracaena deisteliana(Dracaenaceae) ". Mémoire présenté en vue de l'obtention du Diplôme de Professeur de l'Enseignement Secondaire, 2ème Grade, Université de Yaoundé I, Cameroun, 2013.

[13]S. Murugan, D. P. Uma, P. N. Kannika, K.R. MANI. "Antimicrobial activity of SyzygiumJambosagainst selected human pathogens".International Journal of Pharmacy and Pharmaceutical Sciences, vol 3, pp.44-47, 2011.

[14] M.A. Martín, S. Ramos, I. Cordero-Herrero, L. Bravo, L. Goya."Extrait de cacao phénolique protège les cellules bêta du pancréas contre le stress oxydatif". Journal of Nutriments, vol 5, pp. 2955-968, 2013.

[15] G.A. Alitonou, F.P. Tchobo, F. Avlessi, B. Yehouenou, P. Yedomonhan, A.Y. Koudoro, et al. "Chemical and biological investigations of SyzygiumaromaticumL. essential oil from Benin".International Journal of Biological and Chemical Sciences, vol 6, pp. 1360-367, 2012.

[16] S. Boubrit, N. Boussad."Détermination "in vitro" du pouvoir antibactérien des huiles essentielles d'eucalyptus, myrte, clous de girofle et sarriette, et leur application à la conservation de la viande fraîche type hachée". Mémoire du diplôme d'Ingénieur d'Etat en Biologie, option contrôle de la qualité et analyses, Université Mouloud Mammeri de Tizi-ouzou, Algérie, 2007.

[17] F. Chami. "Evaluation in vitro de l'ActionAntifongique des huilesessentiellesd'Origan et de Girofle et de leurs Composés Majoritairesin vivo Application dans la Prophylaxie et le Traitement de la CandidoseVaginalesur des Modèles de Rat et de Souris Immunodéprimés". Thèse de Doctorat, UniversitéSidi Mohamed Ben Abdallah, Maroc, 2005.

[18]L. Jirovetz, G. Buchbauer, I. Stoilova, A. Stoyanova, A. Krastanov, E. Schmidt. "Chemical composition and antioxidant properties of clove leaf essential oil". Journal of Agricultural and Food Chemistry, vol 54, pp. 6303-307, 2006.

[19] G. Razafimamonjison, M. Jahiel, T. Duclos, P. Ramanoelina, F. Fawbush, P. Danthu. "Bud, leaf and stem essential oil composition of Syzygium aromaticum from Madagascar, Indonesia and Zanzibar".International Journal of Basic and Applied Sciences, vol 3, pp. 224-233, 2014.

[20] M. Faucon. Traitéd'aromathérapiescientifique et médicale :fondements& aide à la prescription monographies: huiles essentielles, huiles végétales, hydrolats aromatiques. France. Edition Sang de la Terre,2012, pp. 879.

[21] P. Mailhebiau. La nouvelle aromathérapie: caractérologie des essences ettempéramentshumains. France. Edition Nouvelle Vie, 1989, pp. 372.

[22] J. F. Clevenger. "Apparatus for the determination of volatile oil".Journal of the American

Pharmaceutical Association, vol 17, pp. 346-49, 1928.

[23] P. Mbarga, A.P. Nkoa. "Evaluation de la qualité du beurre de cacao vendudansquelquesmarchés de la ville de Yaoundé". Mémoiresoutenuenvue de l'obtention du Diplôme de l'enseignement technique, 1er grade, ENSET de l'Université de Douala, Cameroun, 2014.

[24] R.P. Adams. "Identification of essential oil components by Gas Chromatography / Mass spectrometry". USA. 4th edition, Allured Publishing Corporation, Carol Stream, 2007, pp. 804.

[25] M.E. Cuvelier, H. Richard, C. Berset. "Comparison of the antioxidative Biochem, antioxydative activity of some acid phénols: Structure-activity relationship". Bioscience, Biotechnology, and Biochemistry journal, vol 56, pp. 324-25, 1992.

[26] P. M. Jazet Dongmo, F. Tchoumbougnang, E. Tchinda Sonwa, F. Fekam Boyom, P. H. Amvam Zollo, C. Menut."Antiradical, antioxidant activities and anti-inflammatory potential of the essential oils of the varieties of citrus limon and citrus aurantifolia growing in Cameroon".Journal of Asian Scientific Research, vol 3, pp. 1046-057, 2013.

[27] P. M. JazetDongmo, F. Tchoumbougnang, B. Ndongson , W. Agwanande, B. Sandjon, P. H. AmvamZollo, et al. "Chemical characterization, antiradical, antioxidant and anti-inflammatorypotential of the essential oils of Canariumschweinfurthiiand Aucoumeaklaineana(Burseraceae) growing in Cameroon". Agriculture and biology journal of north America, vol1, pp. 606-11, 2010.

[28] M. Lahlou. "Methods of studying the phytochemistryand bioactivity of Essential Oil". Journal ofPhytotherapy research, vol 18, pp. 435-48, 2004.

[29] R.F. Houinsou, E.S. Adjou, E.D. Ahoussi, D.C.K. Souhouhloué, M. M. Soumanou. "Biochemical and sensorial charactéristics of cowpea (vignaunguiculata)stored with essentials oils extracted from plant of Myrtaceaefamily". International journal of Innovation and Applied Studies, vol 9, pp. 428-37, 2014.

[30] M.L. Sameza, M.A.B. Boat, C.N.M. Lile, S. Tchameni, M. Nguemnang, B.B. Ampere, et al. Evaluation of clove essential oil as a mycobiocide against Rhizopus stolonifer and fusarium solani, tuber
rot causing fungi in yam (discorea rotumdata). Journal of Phytopathology, pp.8, 2016.

[31] G. Razafimamonjison, M. Jahiel, P. Ramanoelina, F. Fawbush, P. Danthu. "Effects of phenological stages on yield and composition of essential oil of Syzygium aromaticum buds from Madagascar". International Journal of Basic and Applied Sciences, vol 2, pp. 312 – 18, 2013.

[32] P. Eke, W.L. Nana, J. Kuate, B.D. Begoude, T. Tchana, C. Menut, et al. "Antifungal potential of Syzygium aromaticumand Zanthoxylum xanthoxyloides essential oils against Phaeoramularia angolensis causing phaeoramularia leaf and fruit Spot disease on citrus". International Journal of

Current Microbiology and Applied Sciences, vol 4, pp. 964-76, 2015.

[33] V.K. Raïna, S.K. Srivastava, K.K. Aggarwal, S. Ramesh, S. Kumar. "Essential oil composition of CinnamomumzeylanicumBlume leaves from Little Andaman, India". Flavour and Fragrance Journal, vol 16, pp. 374-76, 2001.

[34] K.A.Srivastava, K.S. Srivastava, V. K. Syamsundar. "Bud and leaf essential oil composition of SyzygiumaromaticumfromIndia and Madagascar". Flavour and Fragrance Journal, vol 20, pp. 51-53, 2005.

[35] E.M. Gaydou, R.P. Randriamiharisoa. "Multidimentional analysis of Gas chromatographic data, application to the differenciation of clove bud and clove stem essential oil from Madagascar". Parfumer and Flavorist, vol12, pp. 45-51, 1987.

[36] S. Ali, R. Prasard, A. Mahmood. "Eugenol-rich Fraction of Syzygium aromaticum(clove) Reverses Biochemical and Histopathological Changes in Liver Cirrhosis and Inhibits Hepatic Cell Proliferation". Journal of Cancer Prevention, vol 19, pp.288-300, 2014.

[37] K. Chaieb, T. Zmantar, R. Ksouri, H. Hajlaoui, K. Mahdouani, C. Abdelly, et al. "Antioxidant properties of the essential oil of Eugenia caryophyllata and its antifungal activity against a large number of clinical Candida species". Mycoses, vol50, pp. 403-06, 2007.

[38] T. Atsusane. "Clove oil or dehydroeugenol for controlling oxygen in thehuman body".Japan KokaiTokkyoKoho, vol 227, pp. 6, 1991.

[39]S. Barbelet. "Le giroflier : historique, description et utilisations de la plante et de son huile essentielle". Mémoire de thèse soutenu en vue de l'obtention du diplôme d'Etat de Docteur en Pharmacie, Université de Lorraine, France, 2015.

[40] J.P. Dai, X.F. Zhao, J. Zeng, Q.Y.Wan, J.C. Yang, W.Z. Li, et al."Drug screening for autophagy inhibitors based on the dissociation of Beclin1-Bcl2 complex using BiFC technique and mechanism of eugenol on anti-influenza A virus activity". PLoS One, vol 8, pp.1-9, 2013.

[41] H. Nam, M.M. Kim. "Eugenol with antioxidant activity inhibits MMP-9 related to metastasis in human fibrosarcomacells". Food and chemical toxicology, vol55, pp. 106-12, 2013.